IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Takayuki YAMAZAKI et al.
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For : METHOD FOR TREATING CANCER
Art Unit : Not assigned
Examiner : Not assigned

DECLARATION UNDER 37 C.F.R. 1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I, Kazuyoshi MASAKI, a national of Japan, residing at 345-1, Yokonuma, Sakado-shi, Saitama-ken, Japan, declare as follows.

I am a co-applicant of the above-identified application.

I graduated from University of Tohoku and received Degree of Bachelor in 1981. I have worked for TOYO SUISAN KAISHA, LTD. since April 1981, and I am in Research and Development Department 3rd Section, TOYO SUISAN KAISHA, LTD.

The following Experiments were conducted under my direct supervision.

Experiments

In the Experiments, the following cultured cells and culture mediums were used.

<Cultured Cells>

HeLa : Human cervical adenocarcinoma cells

T24 : Human transitional cell carcinoma; urinary bladder cancer cells (malignant epithelioma)

MCF-7 : Human mammary gland adenocarcinoma cells

SKG-IIIb : Human squamous cell carcinoma; uterine cervix cancer cells

HO-1-N-1 : Human squamous cell carcinoma; oral cancer cells

MIA PaCa-2 : Human pancreatic adenocarcinoma cells

HUH-6 Clone 5 : Human hepatocellular carcinoma cells (malignant epithelioma)

A-172 : Human glioblastoma cells (glioma)

HT-1080 : Human fibrosarcoma cells (sarcoma)

Mewo : Human melanoma cells

U937 : Human lymphoma cells

MOLT-4 : Human leukemia cells

<Culture Medium>

For HeLa : Eagle's minimum essential medium, with 10% of fetal bovine serum, 2g/L of $NaHCO_3$, 60 μg/mL kanamycin added thereto.

For T24 : Eagle's minimum essential medium, with 10% of fetal bovine serum, 2g/L of $NaHCO_3$, 60 μg/mL kanamycin added thereto.

For MCF-7 : RPMI 1640 medium, with 10% of fetal bovine serum, 2g/L of $NaHCO_3$, 25 μg/mL of streptomycin and 25 units/mL of penicillin added thereto.

For SKG-IIIb : Ham's F-12 nutrient mixture, with 10% of fetal bovine serum, 2g/L of $NaHCO_3$.

For HO-1-N-1 : A 1:1 mixture of Dulbecco's modified Eagle's medium and Ham's nutrient mixture F-12, with 10% of fetal bovine serum, 2g/L of $NaHCO_3$.

For MIA PaCa-2 : Eagle's minimum essential medium, with 10% of fetal bovine serum, 2g/L of $NaHCO_3$, 100 μM of non-essential amino acids, 60 μg/mL kanamycin added thereto.

For HUH-6 Clone 5 : Dulbecco's modified Eagle's medium, with 10% of fetal bovine serum, 2g/L of $NaHCO_3$, 60 μg/mL kanamycin added thereto.

For A-172 : Dulbecco's modified Eagle's medium, with 10% of fetal bovine serum, 2g/L of $NaHCO_3$, 60 μg/mL kanamycin added thereto.

For HT-1080 : Eagle's minimum essential medium, with 10% of fetal bovine serum, 2g/L of $NaHCO_3$, 100 μM of non-essential amino acids, 60 μg/mL kanamycin added thereto.

For Mewo : Eagle's minimum essential medium, with 10% of fetal bovine serum, 2g/L of $NaHCO_3$, 100 μM of non-essential amino acids, 60 μg/mL kanamycin added thereto.

For U937 : RPMI 1640 medium, with 10% of fetal bovine serum, 2g/L of $NaHCO_3$, 25 μg/mL of streptomycin and 25 units/mL of penicillin added thereto.

For MOLT-4 : RPMI 1640 medium, with 10% of fetal bovine serum, 2g/L of $NaHCO_3$, 25 μg/mL of streptomycin and 25 units/mL of penicillin added thereto.

Each of compounds of the present invention, SQAG 1 (C14:1), SQAG 2 (C16:1) and SQAG 3 (C18:1), which are identified in Table 1 given in the present specification, page 37, was adjusted to a concentration of 10mg/mL ethanol-decane mixture (49:1) and diluted in the corresponding culture medium. The mixture was added to a 96-well microplate containing the cells at 1 x 10^4 cells/well and the culture was incubated for 48 hours. Then, the MTT assay (Mosmann, T: Journal of Immunological Method, 65, 55-63 (1983)) was carried out and the viability rate of the cells was calculated, from which IC_{50} was determined. The results are shown in Table below.

Table Cytotoxicity against Cancer Cells: IC_{50} (μg/mL)

	SQAG 1	SQAG 2	SQAG 3
HeLa	>100	49	18
T24	>100	33	16
MCF-7	59	17	6
SKG-IIIb	49	13	4
HO-1-N-1	76	30	8
MIA PaCa-2	59	13	6
HUH-6 Clone 5	86	14	7
A-172	48	27	6
HT-1080	30	21	6
Mewo	86	15	4
U937	60	12	4
MOLT-4	64	23	7

The results in the above Table demonstrate that compounds SQAG 1, SQAG 2, and SQAG 3 according to the present invention provide unexpectedly good cytotoxicity against the cancer cells tested.

I, the undersigned, declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Cord and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: Sep 29. 2003

Kazuyoshi Masaki

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